

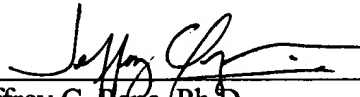
A8 initial 5 min denaturation step at 95°C, *Taq* enzyme was added and thermocycling was performed as above with 30 cycles of denaturation (94°C, 1 min), annealing (55°C or 60°C, 1 min), elongation (72°C, 1 min), followed by a 10 min elongation at 72°C.

REMARKS

The enclosed electronic and paper copies of the Sequence Listing include no new matter that goes beyond the original application as filed, but are supplied to fulfill the requirements as outlined in the Communication from the Examiner. Furthermore, the above amendments, which merely direct the insertion of the Sequence Listing and insertion of sequence identifiers, include no matter that goes beyond the original application as filed. Applicant respectfully submits that the above-identified application is now in compliance with 37 C.F.R. §§ 1.821-1.825.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The first of the attached pages is captioned "Version with Markings to Show Changes Made."

Respectfully submitted,
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 2, line 29, has been amended as follows:

Figure 1. Schematic of the two step overlap extension or crossover PCR protocol used to generate recombinant *S. enteritidis agfA*. (A) Step one of the protocol involved a vector-borne (wavy lines) target *S. enteritidis* gene (grey box) that was PCR amplified in two fragments using two pairs of primers, (A/B, C/D, arrows). Internal primers encoded the foreign epitope (solid black line) whereas external primers encoded restriction endonuclease recognition sites (*Eco*RI or *Hind*III) for subsequent cloning. (B) In step two, both purified PCR products were combined with external primers as described in Experimental procedures and PCR was used to generate gene fragments (1 and 4) which annealed to generate the whole chimeric gene. (C) Recombinant *S. enteritidis agfA* containing 48 bp foreign *Leishmania major* DNA sequence (SEQ ID NO: 9) encoding the 16 amino acid PT3 epitope (SEQ ID NO: 10)[Jardim, 1990]; the protein sequence is indicated in bold type.

Paragraph beginning at page 3, line 20, has been amended as follows:

Figure 3. PT3 epitope replacement in AgfA. A. Peptide sequence of the PT3 epitope from GP63 of *Leishmania major* (SEQ ID NO: 10)[Jardim, 1990]. Predicted secondary structure (Garnier-Robson algorithm, DNASTar software) is listed below the peptide sequence: (-) □-strand ; (x) □-helix. B. Schematic diagram of the mature AgfA protein (SEQ ID NO: 31) illustrating the regions replaced by the PT3 epitope sequence; regions A1 to A10 are indicated with the colored boxes above the sequence. The five-fold internal sequence homology of AgfA is represented by regions C5a-e with the consensus sequence Sx₅QxGx₂NxAx₃Q (SEQ ID NO: 59), with the 22-residue N-terminal region listed at the bottom. Predicted secondary structure (Garnier-

Robson algorithm, DNASTar software) is listed below the protein sequence: (-) \square -strand ; (x) \square -helix ; (*) random coil; (<) turn.

Paragraph beginning at page 5, line 16, has been amended as follows:

Figure 9. AgfA fimbrin domains and internal amino acid sequence homology. (a) Schematic diagram of the N-terminal (N) and C-terminal (C) domains of AgfA illustrating the relative positions of the two- or five-fold homologous regions C2a-b or C5a-e, respectively, within the C-terminus. Values in parenthesis denote the number of amino acids present in each segment. (b) Alignment AgfA (SEQ ID NO: 32) fragments C2a and C2b. Amino acid identity (•) and conservative replacements (underscored) are indicated with gaps (-) introduced for optimal alignments. (c) Alignment of AgfA (SEQ ID NO: 31) fragments C5a to C5e with gaps (-) introduced for optimal alignments. Conserved residues within each repeat are boxed. (d) The 18 amino acid consensus sequence (SEQ ID NO: 33) of the five internal repeats where x is any amino acid. The position of each residue corresponds to the numbered residues in Figure 11c. (e) Position of the conserved ten nonpolar-polar-nonpolar (ifn) triplet motifs within each of the five 22 or 23 residue repeats. Non-conserved residues of each triplet are boxed.

Paragraph beginning at page 5, line 29, has been amended as follows:

Figure 10. Secondary structure predictions of AgfA (SEQ ID NO: 31) in which extended (e), helix (h), coil (c) or turn (t) propensity are noted under each amino acid letter designation. The programs Alexsis (alx), Hierarchical Neural Network (hie), Garnier (gar), Gibrat (gib) and NNpredict (nnp) were used to analyze AgfA as described in the Materials and Methods.

Paragraph beginning at page 6, line 27, has been amended as follows:

Figure 15. Alignment of the five *S. enteritidis* AgfA (SEQ ID NO: 34) C-terminal tandem repeat sequences with those of *E. coli* CsgA (SEQ ID NO: 35) such that the amino acid residue positions are numbered according to Figure 9d and Figure 11c. Symbols above the numbered amino acids indicate the positions of proposed internalized (•), surface exposed (o), turn

(t) residues or the two nonpolar-polar-nonpolar triplets (ifi) within the parallel β helix model of AgfA. Surface or turn residues are colour coded: polar or acidic (red), basic (blue), nonpolar (green), W (yellow) and G (black). Proposed internalized residues are noted in black.

Paragraph beginning at page 7, line 4, has been amended as follows:

Figure 16. Alignments of AgfA with β structural motifs of the three template proteins of known structure used to assemble AgfA models. AgfA sequence was aligned with the (a) β roll motif sequence of *Serratia marcescens* protease (SMP) (SEQ ID NO: 36) (Baumann, 1994; Braunagel & Benedik, 1990), AgfA (SEQ ID NO: 37) (b) β barrel motif of bovine myelin P2 protein (PMP) (SEQ ID NO: 38) (Jones *et al.*, 1988), AgfA (SEQ ID NO: 39), (c) β prism motif of the vitelline membrane outer layer protein I (VMO-I) (SEQ ID Nos: 40-45) (Shimizu & Morikawa, 1996). Bold characters indicate nonpolar-polar-nonpolar motifs, gaps (-) were introduced for optimal alignments. Residues are numbered according to published sequences.

Paragraph beginning at page 7, line 12, has been amended as follows:

Figure 17. Schematic of the two step overlap extension or crossover PCR protocol used to generate chimeric *S. enteritidis* genes. (A) Step one of the protocol involved a plasmid-borne (wavy lines) target *S. enteritidis* gene (grey box) that was PCR amplified in two fragments using two pairs of primers, (A/B, C/D, arrows). Internal primers encoded the foreign epitope (solid black line) whereas external primers encoded restriction endonuclease recognition sites (*Eco*RI or *Hind*III) for subsequent cloning. (B) In step two, both purified PCR products were combined with external primers as described in Experimental procedures and PCR was used to generate gene fragments (1 and 4) which annealed to generate the whole chimeric gene. (C) Chimeric *S. enteritidis* *sefA* and *agfA* fimbrin genes containing 48 bp foreign *Leishmania major* DNA sequence (SEQ ID NO: 9) encoding the 16 amino acid PT3 epitope [25] (SEQ ID NO: 10); the protein sequence is indicated in bold type.

Paragraph beginning at page 9, line 1, has been amended as follows:

Figure 23. PT3 epitope replacement in SefA. A. Peptide sequence of the PT3 epitope from GP63 of *Leishmania major* (SEQ ID No: 10) (Jardim et al., 1990). B. Schematic diagram of the mature SefA protein (SEQ ID NO: 46) illustrating the regions replaced by the PT3 epitope sequence; regions S1 to S10 are indicated with the colored boxes above the sequence. Color Code: Black = region S1; Orange = region S10; Blue = regions S2, S3, S4, S5; Green = regions S6, S7, S8, S9.

Table beginning at page 61, line 4, has been amended as follows:

Table 7. PCR primers used to generate *sefA::PT3* or *agfA::PT3*^a

Primers b	Length	Sequence ^c (5'-3')	SEQ ID NO
14-A	39	TTGGAATTCTTCTTAAATTTTAAAATGGCGTTGAGTAT	47
14-B	78	AGCATGAGCCATTTTCATGTGTAAACAACACGTGTAACGAGCTGA TCATATGCAATAGTAAC CGCTGCCTGAACCACTGC	48
14-C	78	TATGATCAGCTCGTTACACGTGTTGTTACACATGAAATGGCTCA TGCTGGGCCTGCTGTT GCTGCTGGTCAGAAAGTT	49
14-D	39	ATTAAGCTTATACATAATCCCTCTTTAAGTTTTTGCATG	50
17-A	39	GCAGAATTCAGCAGTTGTAGTGCAGAAACAGTCGCATAT	51
17-B	78	TGCATGTGCCATTTTCATGGGTAACAACACGGGTAACCAGCTGA TCATAGTTTTTAGCGTT CCACTGGTCGATGGTGGC	52
17-C	78	TATGATCAGCTGGTTACCCGTGTTGTTACCCATGAAATGGCACA TGCAAAATCAGACCGCA TCTGATTCCAGCGTAATG	53
17-D	39	AGACGCAAGCTTCGTTTAATGTGACCTGAGGGATCACCG	54

^a Primers used to generate *sefA::PT3* or *agfA::PT3* as noted in Fig 17.

^b Primers prefixed with 14- or 17- used to generate *sefA* (SEF14) or *agfA* (SEF17) recombinants, respectively.

^a Underlined sequence corresponds to the 48 bp PT3 DNA sequence; bold letters correspond to *Eco*RI (GAATTC) or *Hind*III (AAGCTT) restriction endonuclease sites.

Paragraph beginning at page 61, line 13, has been amended as follows:

To amplify regions surrounding *sefA* and *agfA* in Ap^S *S. enteritidis* strains after gene replacement, primers IN1 (5'-GGG ATG TTG TGT AAA GAT AAA AAA ATA GTG-3') ([SEQ ID NO: 55](#)) and IN2 (5'-TGC CCA ATC TTA GGC CAT AAT ATT TTT GTG-3') ([SEQ ID NO: 56](#)) or TAF59 (5'-AGG AAG GAT CAA AAC TAT TGT CCG TTA TTT CAC-3') ([SEQ ID NO:](#)

57) and TAF60 (5'-TAT ATT TAC ACT AAG ACG AGA CAA CTC AAT CGG-3') (SEQ ID NO: 58) were used, respectively. To obtain template DNA for each *S. enteritidis* strain sequenced, cells from a 1 mL overnight LB culture were harvested and boiled for 10 min. in 1 mL dH₂O. 100 µl PCR reactions contained 20 µl of the boiled whole cell supernatant, 50 pmol of each primer, 0.2 mM of each deoxynucleotide triphosphate and 4 U of *Taq* DNA polymerase (Boehringer Mannheim) in buffer supplied by the manufacturer. After an initial 5 min denaturation step at 95°C, *Taq* enzyme was added and thermocycling was performed as above with 30 cycles of denaturation (94°C, 1 min), annealing (55°C or 60°C, 1 min), elongation (72°C, 1 min), followed by a 10 min elongation at 72°C.